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ASSISTED REPRODUCTION TECHNOLOGIES

Agonist depot versus OCP programming of frozen embryo transfer: a retrospective analysis of freeze-all cycles

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Abstract
Purpose In segmented ART treatment or so-called 'freeze-all' strategy fresh embryo transfer is deferred, embryos cryopreserved, and the embryo transferred in a subsequent frozen embryo transfer (FET) cycle. The purpose of this cohort study was to compare a GnRHa depot with an oral contraceptive pill (OCP) programming protocol for the scheduling of an artificial cycle FET (AC-FET) after oocyte pick-up (OPU).
Methods This retrospective cohort study was conducted on prospectively performed segmented ART cycles performed between September 2014 and April 2015. The pregnancy, treatment duration, and cycle cancellation outcomes of 170 OCP programmed AC-FET cycles were compared with 241 GnRHa depot programmed AC-FET cycles.
Results No significant difference was observed in the per transfer pregnancy and clinical pregnancy rates between the OCP and GnRHa groups, 72.0 versus 77.2 %, and 57.8 versus 64.3 %, respectively. Furthermore, the early pregnancy loss rate was non-significantly different between the OCP and GnRH protocol groups, 19.8 versus 16.7 %, respectively. However, nine (5.29 %) cycles were cancelled due to high progesterone in the OCP protocol group, while no cycles were cancelled in the GnRHa protocol group and the time taken between OPU and FET was 19 days longer (54.7 vs 35.6 days) in the OCP protocol compared to the GnRHa protocol.
Conclusions The results of this AC-FET programming study suggests that the inclusion of GnRHa depot cycle programming into a segmented ART treatment will ensure pregnancy, while significantly reducing treatment duration and cycle cancellation.

Keywords Freeze-all · FET · Artificial cycle · GnRHa · OCP · Programming

Capsule No significant differences were observed in the per transfer pregnancy rates and early pregnancy loss rates when either OCP or GnRHa was used to program FET.

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Introduction

Over the last decade, there has been an increase in the use of frozen embryo transfers (FET) in assisted reproductive technology (ART) programs [1]. Concomitantly, significant improvements have been made to cryopreservation technology, resulting in improved freeze-thaw outcomes from FET [1, 2]. The resultant confidence in FET has encouraged ART centers to more readily consider routine cohort cryopreservation [3, 4]. The rationale to use so-called segmented-ART treatment has been the avoidance of iatrogenic complications related to controlled ovarian stimulation (COS), such as, ovarian hyperstimulation syndrome (OHSS) and premature progesterone rise [3, 5]. In the latter case, deferring fresh transfer, freezing all embryos, and transferring frozen-thawed embryos in a subsequent physiologically normal cycle has been shown to potentially result in improved embryo implantation and placentation and consequently improved perinatal and neonatal outcomes [1–3, 6–13]. However, segmented treatment also has complications, i.e., the management of an increased number of confounding variables and an increase in the treatment duration. Before implementing segmented treatment, it would, therefore, be wise to ensure that all the elements in the

segmented treatment process, i.e., cycle programming, COS, oocyte pickup, embryo culture, embryo cryopreservation, FET cycle programming, FET cycle management, embryo thawing, embryo transfer, and luteal phase support, are cost-effectively managed, produce controlled outcomes, and satisfy patient expectations.

In the past, advice to patients with regard to the start of further treatment (i.e., COS and fresh ET or FET) after a failed IVF cycle was to preferably wait for their second menses, however, a recent publication did not find any differences in reproductive outcomes related to menses number [14]. If this advice is followed in segmented treatment, the total treatment period would be approximately 76 days in length. In 2015, we reported that blastocyst FET results in better pregnancy, implantation, and fetal growth outcomes as compared to fresh blastocyst ET [15], after which we decided to implement a routine segmented treatment program. As the individual protocols for fresh blastocyst ET and frozen-thawed blastocyst ET had proven optimal outcomes, the only concerns were patient related issues (i.e., the length of treatment and the predictability of treatment). Based on these concerns, it was decided to continue the use of artificial cycle (AC) FET for all cycles, even though natural cycle (NC) FET could be considered to be simpler it would, however, decrease predictability—even in the regularly cycling patient—and increase patient clinic interaction. Importantly, in a recent meta-analysis, Groenewoud et al. [16] found that all types of FET protocols (i.e., NC, modified-NC, AC, and modified-AC) were equally successful in terms of ongoing pregnancy.

The only element of segmented treatment that in our hands still required validation was a cycle programming protocol to not only clinically connect the freeze-all cycle with the AC-FET cycle, but also maintain patient engagement for the duration of treatment. In IVF, a number of cycle programming options have been investigated for scheduling of COS and subsequent fresh transfer, all using hormonal pre-treatments of varying duration [17]. The oral contraceptive pill (OCP) has been the most popular of these options and continues to be so notwithstanding some controversy around the possible negative effect OCP may have on ongoing pregnancy outcomes [18]. In this retrospective cohort study, we compared the outcomes of two different FET cycle programming protocols, both starting immediately after OPU. One protocol used OCP and the other a GnRHa depot injection for programming.

Materials and methods

This retrospective single-center cohort study was conducted in prospectively performed segmented ART cycles, performed from September 2014 to April 2015, at a private ART center. Patients, as a routine, provide informed consent for the use of their clinical data in research. Patients who refuse consent are excluded from studies. An Institutional Review Board and Ethics Committee of the (reference no. 443b/2014) approved the design and methodology of the study. The patient cycles were extracted without patient identifiers from the clinic's ART database, with desciptive characteristics detailed in Table 1. The analyses were performed on the outcomes of the freeze-all cycles and the outcomes of only the 1st subsequent FET cycles. Patients were only included once in the analysis and only vitrified-warmed blastocysts were transferred. The two cycle programming protocols illustrated in Table 3 were used: in the GnRHa protocol the patients received a single Lucrin depot injection (3.75 mg, Leuprorelin acetate, Abbott, Mumbai, India) and in the OCP protocol the patients used OCP (Ginera®, 75 μg Gestodene, 30 μg ethinyloestradiol, Bayer, Istanbul, Turkey) daily. For further details see the artificial cycle programming section.

Controlled ovarian stimulation, oocyte pickup, and embryo culture

COS was performed using a GnRH antagonist (Cetrotide, 0.25 mg, Merck Serono, Istanbul, Turkey) co-treatment protocol with a combination of rFSH (150–375 IU, Gonal-F, Merck Serono, Istanbul, Turkey) and hMG (75–150 IU, Menopur, Ferring Pharmaceuticals, Mumbai, India). A transvaginal ultrasound-guided OPU procedure was performed 36 h after ovulation induction with a bolus of GnRHa (0.2 mg, Gonapeptyl®, Ferring Pharmaceuticals, Mumbai, India), when at least three follicles reached 17 mm. Oocyte collection and embryo culture were performed using Sydney IVF media (COOK Medical, Limerick, Ireland). All inseminations were performed using ICSI. Incubation conditions were set at 6 % CO_2, 5 % O_2, and 37.0 °C (K-Systems, Kivex Biotec Ltd, Birkerod, Denmark) and embryos cultured in microdroplets of media overlayed with light mineral oil. All embryos were cultured to at least the sixth day from the day of OPU for cryopreservation.

Blastocysts were scored according to the three-part grading system proposed by Gardner and Schoolcraft [19, 20]; blastocyst expansion on a scale of 1 to 6, the inner cell mass (ICM) on a scale of A to C, according to the number and degree of compaction of the cells, and the trophectoderm (TE) on a scale of A to C, according to the number, size, shape, and contiguous arrangement of the trophectoderm cells. Blastocysts with expansion grade ≥1 and inner cell mass and trophectoderm scores of A or B were cryopreserved by vitrification.

Blastocyst vitrification and warming

Vitrification and warming of blastocysts was performed as described in the manufacturer's methodology insert (Cryotop, Kitazato BioPharma Co. Ltd). The method utilizes high concentration cryoprotectants with ultra-rapid

Table 1 Patient demographics and ICSI treatment cycle outcomes

	OCP group n = 170	GnRHa group n = 241	*p* value	95 % CI
Patient demographics				
Female age	32.2 (5.53) years	32.1 (5.79) years	NS	−1.219–1.019
BMI	26.0 (4.84) kg/m^2	25.6 (4.99) kg/m^2	NS	1.370–0.570
Parity	0.27 (0.59)	0.21 (0.47)	NS	−0.163–0.043
Total AFC	17.3 (12.4)	17.8 (13.1)	NS	−2.020–3.020
Infertility duration	5.17 (3.85) years	5.63 (4.37) years	NS	−0.360–1.280
COS and in vitro outcomes				
COS duration	9.3 (2.30) days	9.1 (1.70) days	NS	−0.588–0.188
Total FSH	3541.8 (1097.3) IU	3285.2 (1131.6) IU	0.022	−476.6–36.70
Total oocytes	17.2 (9.94)	17.5 (11.51)	NS	−1.844–2.444
Blastocyst number[a]	4.4 (3.09)	4.6 (3.56)	NS	−0.464–0.864
Blastocyst utilization[b]	46.2 (22.1)	49.6 (24.8)	NS	−1.270–8.070

NS not significant, *BMI* body mass index, *AFC* antral follicle count, *COS* controlled ovarian stimulation, *FSH* follicle stimulating hormone

[a] Blastocyst number; the mean number of blastocysts vitrified per cycle

[b] Blastocyst utilization; the ratio of total blastocyst number to total 2PN number

vitrification and warming rates. Equilibration, vitrification, thawing, diluent, and washing solutions required for the procedures are provided in the commercially available Cryotop Safety Kits. Briefly, blastocysts were taken through an equilibration step and a vitrification step at room temperature before being placed on a Cryotop strip and plunged into liquid nitrogen. No more than two blastocysts were placed on a strip. The Cryotop strip was placed in a cap under liquid nitrogen and stored in a cryotank. For warming, a capped Cryotop container was removed from the cryotank and the strip removed from the cap under liquid nitrogen. The strip was taken from the liquid nitrogen and plunged into warming solution. Recovered blastocysts were taken through a diluent and two wash steps at 37 °C. After the last wash step, the blastocysts were placed in culture droplets covered with light mineral oil and placed in an incubator for an equilibration period of at least 2 h. After the equilibration period, the blastocysts were again assessed for survival and rescored. The selected blastocyst(s) was transferred to a transfer dish and returned to the incubator, while all other surviving blastocysts were revitrified.

Cycle programming

The GnRHa and the OCP protocols used to program the start of the endometrial preparation are illustrated in Table 3. In the OCP programming protocol, OCP administration was started 10 days after OPU and continued for at least 21 days. Endometrial preparation commenced 5 days after the last pill—wash-out—(35 days after OPU) and the transfer was performed on day 20 of endometrial preparation (54 days after OPU). In the GnRHa depot programming protocol, half a dose (≈1.88 mg) of Lucrin depot [21] was administered 7 days after OPU. Endometrial preparation was started 10 days later (17 days after OPU) and the transfer was performed on day 20 of endometrial preparation (36 days after OPU).

Endometrial preparation

The same endometrial preparation protocol was used in both groups. Estrogen (Estrofem, Novo Nordisk, Istanbul, Turkey) was taken orally in a step-up regimen, 2 mg/day from day 1 to 6, 4 mg/day from day 7 to 10, and 8 mg/day from day 11 to 14. On the 14th day of estrogen therapy a transvaginal ultrasound scan (TVUS) was performed to measure endometrial thickness. An endometrial thickness of >7 mm was considered most optimal [16, 22]. On the day of scan, serum progesterone was also measured, with FET cycles cancelled if the serum progesterone concentration was above an arbitrary chosen threshold (2 ng/mL). If the endometrium was >7 mm and there was no evidence of a rise in progesterone, estrogen supplementation was continued at 6 mg/day, and progesterone (8 % twice a day, Crinone, Merck Serono, Turkey) supplementation commenced the following day (day 15), and the vitrified-warmed blastocyst transfer was performed on the sixth day of progesterone therapy. The daily dose combination of estrogen (Estrofem, 6 mg/day) and progesterone (Crinone, 8 % BD) continued for the duration of the luteal support—until at least 12 weeks of gestation.

Frozen embryo transfer

All blastocyst transfer procedures were performed using a Hamilton syringe (50 μL, Hamilton syringe, Sigma-Aldrich, St Louis, MO, USA) attached to an embryo transfer catheter (Wallace, Smiths Medical International, Kent, UK) and transabdominal ultrasound guidance.

Outcome measures

The primary outcome measures used to compare the outcomes in the GnRHa and OCP protocol groups were the following: biochemical pregnancy, i.e., a day 14 βhCG concentration of ≥30 IU/L, clinical pregnancy, i.e., an intra- or extrauterine fetal heart beat confirmed by ultrasound, treatment duration, i.e., the time from OPU to vitrified-warmed blastocyst transfer, and cycle cancellation, i.e., cycles with an serum progesterone concentrations greater than an arbitrary chosen threshold of 2 ng/mL.

Statistical analysis

MedCalc version 13.0.6 was used for statistical analysis and to obtain the confidence intervals and risk ratios. Descriptive statistics were presented as the mean and standard deviation for continuous data and as percentages for the categorical data. The independent samples *t* test was used to compare the means, and the chi-square or the Fisher exact test was used to determine statistical significance between percentages. $p < 0.05$ as considered to be significant.

Results

Patient and treatment covariables

In total, 411 of the segmented treatment cycles completed during the study period were included in the analysis. In 170 cycles, ICSI, freeze-all, OCP programming, and AC-FET were performed and in 241 cycles ICSI, freeze-all, GnRHa depot programming, and AC-FET were performed. The patient baseline characteristics and the COS, ICSI, and freeze-all outcomes are presented in Table 1. All the characteristics and the outcomes were non-significantly different between the two groups, except for the total number of FSH units used, which was significantly higher in the OCP protocol group (Table 1). The longer duration and higher number of FSH units, however, resulted in the total oocyte number (17.2 vs 17.5) being comparable between the two groups (Table 1). In both protocol groups, the main indications for fertility treatment were unexplained infertility and male infertility. In the OCP protocol group, unexplained infertility was 26.5 % and male infertility 24.7 %, while in the GnRHa protocol group the proportions were 23.7 and 28.2 %, respectively. These proportions were comparable between the two protocol groups, with *p* values of 0.498 and 0.514, respectively.

Cycle cancellation

Of the 170 AC-FET cycles started with OCP programming, nine (5.3 %) were cancelled before the day of transfer. In the GnRHa protocol group, no cycles (0 %) were cancelled (Table 2). The reason for cycle cancellations in the OCP protocol was progesterone rise (>2 ng/mL). No cycles were cancelled, because of of blastocyst non-survival or a thin endometrium. The blastocyst survival rate was 97.7 % (250/256) in the OCP protocol group and 97.0 % (384/396) in the GnRHa protocol group. All surviving blastocysts that were not selected for transfer were re-vitrified.

Endometrial thickness, progesterone concentration, and treatment duration

The proportions of thin endometrium (<7 mm) cycles were comparable between the two groups, 2.94 % in the OCP protocol group and 2.49 % in the GnRHa protocol group (Table 2). In total, 11 cycles had a thin endometrium. All cycles with thin endometria continued with treatment, on clinical assessment (i.e., patient etiology and previous treatment history), with no added days of endometrial preparation. In 8 of the 11 (72.7 %) cycles, a pregnancy was obtained, with 4.5 mm being the thinnest endometrium with a pregnancy. There was no statistical difference between the two groups in terms of day 14 endometrium thickness (10.0 mm vs 9.90 mm) per completed cycle (Table 2). In the OCP protocol group there were 25 cycles with a progesterone concentration of >1 ng/mL, with 9 cycles having a concentration of >2 ng/mL. The proportion of cycles with an elevated progesterone concentration (>1 ng/mL) in the OCP protocol group was statistically higher than the proportion in the GnRHa protocol group, 14.7 versus 6.2 %, respectively (Table 2). There were no cycles in the GnRHa protocol group that had a progesterone concentration >2 ng/mL. The per transfer pregnancy rate for cycles with a progesterone concentration of >1 ng/mL in the two protocol groups was 56.2 % (9/16) and 66.7 % (10/15), respectively.

The time period between OPU and FET was approximately 19 days longer in the OCP protocol group (54.7 vs 35.6 days). Not all cycles included in the study adhered precisely to the programming protocols illustrated in Table 3. In the OCP protocol, 17 patients used OCP for only 20 days, with a pregnancy rate of 64.7 % and 70 used OCP for 22 to 25 days, with a pregnancy rate of 68.5 %. In the GnRHa protocol group, 154 patients started endometrial preparation 9 days after the GnRHa depot injection, with a pregnancy rate of 77.9 % and 53

Table 2 Vitrified-warmed blastocyst FET cycle outcomes

		OCP group n = 170	GnRHa group n = 241	*p* value	RR	95 % CI
Cycle cancellation % (*n*)[a]		5.29 (9/170)	0 (0/241)	0.002	26.9	1.576–458.9
FET cycles (*N*)		161	241			
Blastocysts transferred	Mean (std)	1.30 (0.46)	1.32 (0.47)	NS		−0.071–0.111
Endometrial thickness	Mean (std)	10.0 (2.32) mm	9.90 (1.86) mm	NS		−0.506–0.306
Thin endometrium[b]	% (*n*)	2.94 (5/170)	2.49 (6/241)	NS	1.18	0.367–3.808
Progesterone concentration	Mean (std)	0.57 (0.35) ng/mL	0.477 (0.28) ng/mL	0.003		−0.154–0.032
Progesterone ≥1 ng/mL[c]		14.7 (25/170)[d]	6.2 (15/241)	0.007	2.36	1.285–4.345
OPU to FET[e]	Mean (std) Minimum (range)	54.7 (1.28) days 52 (52–58) days	35.6 (0.83) days 35 (35–37) days			
Pregnancy outcomes						
Pregnancy per transfer	% (*n*)	72.0 (116/161)	77.2 (186/241)	NS	0.93	0.830–1.051
Pregnancy per cycle	% (*n*)	68.2 (116/170)	77.2 (186/241)	NS	0.88	0.782–1.000
Clinical pregnancy per transfer[f]	% (*n*)	57.8 (93/161)	64.3 (155/241)	NS	0.90	0.764–1.056
Chemical pregnancy loss	% (*n*)	8.62 (10/116)	8.60 (16/186)	NS	1.00	0.471–2.133

NS, Not significant, *OPU*; Oocyte pickup, *FET*; Frozen embryo transfer

[a] Cycle cancellation; cycles with progesterone concentration levels of >2 ng/mL were cancelled

[b] Thin endometrium; the proportion of FET cycles with endometrial thickness of <7.0 mm

[c] Progesterone ≥1 ng/mL; the proportion of FET cycles with a progesterone ≥1 ng/mL

[d] Includes 16 cycles with a progesterone concentration of 1–2 ng/mL and nine with a concentration of >2 ng/mL

[e] OPU to FET; the number of days from the day of OPU to the day of FET

[f] Clinical pregnancy per transfer; ultrasound confirmed fetal heart per transfer

started 11 days after the depot injection, with a pregnancy rate of 71.7 %.

Reproductive outcomes

The number of blastocysts per transfer was comparable between the two groups, 1.3 versus 1.32, respectively. Moreover, there was no significant difference in terms of pregnancy between the two groups, i.e., pregnancy rate per transfer (72.0 vs 77.2 %) pregnancy rate per started cycle (68.2 vs 77.2 %) and clinical pregnancy per transfer (57.8 vs 64.3 %) (Table 2). The total early pregnancy loss rates (19.8 vs 16.7 %, $p = 0.587$), and the chemical pregnancy loss rates (8.62 vs 8.60 %) were also comparable between the two groups.

Discussion

The results from the analysis of the 411 segmented ART cycles in this analysis showed no difference in clinical pregnancy and early pregnancy loss rates between AC-FET cycles programmed with either OCP or GnRHa depot. In the study analysis, there were three measures that were significantly different between the two protocols, cycle cancellation (5.3 vs 0 %) based on an arbitrary serum progesterone threshold of 2 ng/mL, treatment duration, and total FSH dose. While, the former two were of significance to the study outcomes, the later was regarded as a random difference, as oocyte number a primary indicator of ovarian response was non-significantly different, as well as, the per transfer pregnancy rates—an indicator of endometrial receptivity. The primary objective of the study was to investigate the efficiency and effectivity of two AC-FET cycle scheduling protocols that would connect the freeze-all with the AC-FET cycle in segmented ART. The other elements that make-up a segmented ART cycle, i.e., GnRH antagonist co-treatment COS with GnRH agonist trigger, OPU, ICSI, blastocyst culture, blastocyst vitrification, and AC-FET, were all previously implemented and resulted in an overall AC-FET pregnancy rate of approximately 73 % from the transfer of 1.28 blastocysts per transfer.

AC-FET is the standard protocol used at our center for frozen-thawed embryo transfers, due to its low-monitoring requirement, its predictability (i.e., determinable embryo transfer date) and its relatively low cancellation risk. In a recent review and meta-analysis, no single endometrial preparation method for FET was found to be superior, with all methods being equally effective in terms of ongoing pregnancy [16]. The connecting protocol, therefore, needs to maintain the existing cost-effectivity and predictability of treatment without compromising reproductive outcome. Moreover, the connecting protocol should not increase patient stress and discomfort levels.

OCP and GnRHa are both suitable candidates to program the start of an AC-FET cycle, due to their inherent

Table 3 The programming of FET following ICSI treatment in freeze-all cycles

	OPU	Days of blastocyst vitrification	OCP	OCP wash-out	Endometrial preparation				E	FET
					E 2 mg	E 6 mg	E 8 mg	Day 14 X_{ET} X_P	6 mg P 8% × 2	
The number of days	1	6	7	10–30	35–40	41–44	45–48	48	49–54	54
	OPU	**Days of blastocyst vitrification**	**Downregulation period**		**Endometrial preparation**				**E**	**FET**
		Lucrin depot			**E 2 mg**	**E 6 mg**	**E 8 mg**	**Day 14 X_{ET} X_P**	**6 mg P 8% × 2**	
The number of days	1	6	7		17–22	23–26	27–30	30	31–36	36

OPU oocyte pickup, *FET* frozen embryo transfer, *E* estrogen supplementation (2 mg, Estrofem, Novo Nordisk, Istanbul, Turkey), *P* progesterone supplementation (8 %, Crinone, Merck Serono, Turkey), *OCP* oral contraceptive pill (Ginera®, Bayer, Istanbul, Turkey) for at least 21 days

Vitrification; blastocyst vitrification using the Cryotop method

Lucrin depot; ½ dose (3.75 mg, Leuprorelin acetate, Abbott, Mumbai, India) bolus injection

$X_{ET,}$ TVUS to measure endometrial thickness, with >6 mm preferred

$X_{P,}$ Progesterone blood concentration, with <2 ng/mL preferred

characteristics. The OCP and GnRHa programming schedules used in the study are illustrated in Table 3. The minimum number of days from OPU to FET was 54 in the OCP protocol and only 36 days in the GnRHa protocol. Lucrin depot was preferentially chosen for its single dose application in this study. The depot dose was also halved (1.88 mg) to moderate pituitary suppression and prevents possible hypoestrogenic side-effects [21]. In the OCP protocol, the standard administration duration of 21 days was chosen to ensure adequate suppression, as the level of pituitary and ovarian suppression was found to be related to the length of OCP administration [23]. In both protocols, the programming of the AC-FET cycle was started within the freeze-all cycle. In the OCP protocol, OCP administration started 10 days after OPU, and in the GnRHa protocol, the GnRHa bolus was administered 7 days after OPU.

Importantly, OCP and GnRHa programming should support pituitary and ovarian suppression for the duration of the endometrial preparation and, therefore, prevent a rise in progesterone that might compromise embryo-endometrial synchrony. In the OCP programmed cycles of the present study, there was a significantly higher risk (RR = 2.36) for a ≥1 ng/mL rise in progesterone. Moreover, in 9 cycles (5.3 %), the rise was ≥2 ng/mL, resulting in cycle cancellation. The rise in progesterone levels occurred, notwithstanding, the 21-day OCP administration period. In contrast, no cycles using GnRHa programming were cancelled. In the study by Van de Vijver et al. [22], comparing cancellation rates in AC-FET with and without GnRHa downregulation, they obtained cancellation rates of 0 and 1.9 %, respectively, using a 1.5 ng/mL progesterone threshold. In IVF with fresh ET studies, a rise in progesterone at the end of the follicular phase was found to result in reduced reproductive outcomes [5, 24]. In the study by Labarta et al. [24], the expression of genes involved in endometrial maturation and implantation were also observed to be dysregulated in cycles with elevated progesterone concentrations (>1.5 ng/mL). A similar effect of progesterone on reproductive outcomes was observed in the present study, as reduced reproductive outcomes were seen in cycles with progesterone levels of 1 to 2 ng/mL. It is, therefore, of clinical importance to select processes and procedures in segmented ART that limit any transient rise in progesterone during endometrial preparation.

Cycle programming protocols have the potential to not only suppress pituitary and ovarian activity, but also to affect endometrial preparation and ultimately reproductive outcomes. In the present study, there was no difference in mean endometrial thickness or the proportion of cycles with thin endometria, between the two protocols after 14 days of estrogen medication. This was in contrast to the results of a study investigating the effect of OCP pretreatment in GnRH antagonist co-treatment cycles. In OCP programmed cycles, the endometrial thickness was on average lower in young (≤35 years) and old (≥36 years) patients [25]. Cycles with endometrial thicknesses of less than ≤6 mm have generally been cancelled, as they are regarded to be reproductively insufficient. However, in the present study as in the study by Dain et al. [26], thin endometria were seen to not necessarily exclude pregnancy. Moderating factors might be the daily dose of estrogen and the duration of the endometrial preparation. In our study, all patients followed the same step-up estrogen regimen (2 mg to 8 mg/day) for a total of 14 days.

In the present study, the pregnancy rate per started cycle was non-significantly lower in the OCP protocol group (68.2 vs 77.2 %). It is, however, uncertain whether the reduced (Δ 9.0 %) pregnancy rate observed in the OCP protocol group

was the direct result of OCP use [18], or only indirectly, as the result of cycles with elevated progesterone levels. This question requires further investigation. Importantly, it was also recently shown that OCP pretreatment to start GnRHa cotreatment controlled ovarian stimulation for IVF had no negative effect on the genes normally expressed during the 'window of implantation' [23]. Therefore, one would theoretically expect OCP pretreatment to have no negative impact on endometrial receptivity and embryo implantation.

Even though the GnRHa protocol did not result in a statistically significant benefit in terms of pregnancy, other outcomes like cycle cancellation and duration significantly favored its implementation in our segmented ART program, over the OCP protocol. While, cycle cancellation is an important consideration for patients in accepting segmented ART, the potential to have no cancellations is also of value to the clinic. Under such conditions of certainty, clinicians are able to provide patients, on the day of OPU, with the exact dates for their FET procedures and clinics can optimally manage their workload schedules. The short duration (17 vs 35 days) between OPU and the start of endometrial preparation may also limit any feeling of disconnect a patient may experience between the OPU and FET procedures (Table 3).

A limitation of the present study is that even though cycle data was collected prospectively, the study was not a randomized controlled trial and, therefore, suffers from biases inherent to retrospective studies. Moreover, the study was under powered on sample size to show statistical significance between reproductive outcomes at the pregnancy levels observed. The hypothesis was, however, accepted on the other primary measures, i.e., a significantly shorter time period between OPU and FET, and a significantly lower cancellation rate. Of particular importance is the fact that when the GnRHa protocol is used the transfer date can be determined on the day of ovulation induction with great accuracy—an outcome that benefits the clinic and the patient.

All considered, the results of this FET programming study suggest that with the inclusion of the GnRHa depot protocol all the elements are in place to provide a segmented ART treatment balanced in terms of cost-effectiveness, efficiency, success, and risk. However, if FET is going to replace fresh ET universally for reasons of better peri-implantation outcomes [13], we will first have to address the concerns raised in a number of perinatal studies reporting large for gestational age, extreme preterm birth, and vascular dysfunction in off-spring conceived after FET [27–29]. Even with the reassurance that other FET perinatal outcomes are more optimal than after fresh ET and very similar to those of natural conceptions, further studies are necessary to investigate the possible mechanisms involved in the epigenetic modifications that might result from extended embryo culture and cryopreservation.

Compliance with ethical standards

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